A Note on the Effect of Acid, Alkali, and certain Indicators in Arresting or otherwise Influencing the Development of the Eggs of Pleuronectes platessa *and* Echinus esculentus.

By Edward Whitley, M.A. (Oxon.).

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I. Effect of Acid and Alkali on the Eggs of Pleuronectes.

While working last spring at the Port Erin Biological Station on the effect of acids and alkalies upon the development of Echinus eggs, it was suggested to me that it might be interesting to try the general effect of similar solutions upon some other type of organism.

For this purpose the eggs of the Plaice (*Pleuronectes platessa*) were selected, as they were to be obtained in abundance from the fish-hatchery attached to the station, but, as time pressed, it was only found possible to experiment with one acid and one alkali, and decinormal solutions of hydrochloric acid and sodium hydrate were accordingly used.

In a pond attached to the hatchery numbers of plaice are kept in the spawning season, and the surface of the pond is skimmed each morning for the purpose of collecting the eggs, which are then placed in the hatching apparatus. Three batches of these eggs of different ages were taken for experimentation—those freshly skimmed from the pond, those which had been removed two days before and had remained since in the hatching apparatus, and those taken 10 days before and similarly treated. Some few eggs always escape the net in the process of skimming, and are taken in the catch of later days, so that, when 10 days old eggs, for instance, are spoken of, what is meant is that none can be younger than that, although some few may be slightly older. Such as showed obvious differences in age from the majority of each batch were removed.

The eggs were treated in a similar way to that employed in a research carried out at the same time on Echinus eggs,* namely, they were placed in small batches in a number of tumblers, each containing 200 c.c. of sea-water, to which measured amounts of the decinormal solutions of acid or alkali were added. A summary of previous work on the effects of acid and alkaline solutions upon development will be found in the paper above referred to.

* See preceding paper.

The accelerating effect of small quantities of alkali on growth observed by Loeb in the case of Tubularia,* and the eggs of Arbacia,† and in those of Echinus, in the research mentioned above, was not noticed, but attention may be drawn to the very powerful results of even a small disturbance of the chemical equilibrium, which can be seen from Table I, where it will be observed that, after six days, 4 c.c. of decinormal acid or alkali in 200 c.c. of sea-water (*i.e.*, a five-hundredth normal solution) produces a death-rate among fresh eggs of 75 and 44 per cent. respectively, against only 5 per cent. in the Control.

In the experiments above alluded to with the eggs of *Echinus esculentus*, it was found that acids and acid salts above a very small concentration produced more deadly effects than corresponding quantities of alkalies and alkaline salts, and this was also found to be the case with Pleuronectes, and can be well seen in the same table (I) for strengths of 2·5 c.c. and upwards, especially with the younger eggs.

Probably three factors enter into the explanation :—

(1) The fact that part of the alkali added is immediately thrown out of solution as insoluble hydrates or carbonates.

(2) Alkali is constantly being used up to neutralise the acid products of metabolism—chiefly CO_2.

(3) According to Loeb, the presence of weak alkali assists the absorption of oxygen by the organism. If this be the case, the eggs in the acid solutions not being able to absorb oxygen so readily as the others, might probably be less resistant to the action of the reagent.

The tables seem also to show conclusively that the younger eggs are far more sensitive to the action of the acid or alkali, and generally to the influences of their environment than are the older eggs, or newly-hatched larvæ, which are, indeed, extraordinarily resistant. Thus, on referring again to Table I, it will be seen that a very large percentage of the older eggs survived 7, and even 11, days' treatment, whereas, of the fresh eggs, over 25 per cent. in the Control, and a much larger number in all the other cases were dead within 10 days. In this table the effect only of very small quantities of acid or alkali is given (five-hundredth normal and under), but experimentation with somewhat larger amounts gave the same result as shown in Table II. Thus, it needed only 6 c.c. of decinormal sodium hydrate to kill all the fresh eggs in four days, but more than 8 c.c. to kill all those of two days old in the same time, while, by the end of that period, 30 c.c. of alkali

* 'Univ. of California Publications, Physiol.,' vol. 1, 1904, p. 137, and 'Arch. f. gesant. Physiol.,' vol. 101, 1904, p. 340.

† 'Arch. f. Entwickelungsmechanik,' vol. 7, p. 631.

which was the greatest strength employed, had killed only 65 per cent. of the 10-days-old eggs. Acids, above a small concentration, had a remarkably stronger effect—in one day even 10-days-old eggs being killed by 10 c.c., and the two other batches by 6 c.c.

When working with the larger quantities of alkalies, it was very difficult to tell exactly when an egg was dead. In the first experiments the point was taken at which the egg began to become opaque, but, as soon as the percentage of alkali present exceeds a very small amount, precipitation of calcium and magnesium hydrates takes place, which renders observation of such a change in the transparency of the egg difficult. Loeb, in his experiments on Fundulus,* finds that the precipitate itself acts injuriously upon the eggs, and that, if this be filtered off, the eggs will live and develop in much stronger solutions than they would otherwise do. The process of filtering off the precipitate before placing the eggs in the solutions was not tried in the course of these experiments, they having been carried out previously to the reading of Loeb's paper.

Hitherto, it has always been considered that the young larva, on first hatching from the egg, enters upon the most critical stages in its career, and is at that time most susceptible to external influences, but the experiments here carried out appear to show a resistance steadily increasing with age, and that, even after the rupture of the egg-capsule, the young larva is, at all events, no more susceptible than just before that event. It may be suggested that this steadily increasing resistance is due to the gradual development of the epidermal cells, which would form a protection to the young embryo more or less impervious to the surrounding solution.

Tables III, IV, and V give the actual experimental data on which Table I is founded, and of which it is a *résumé*. Table VI is introduced with the object of laying emphasis on the statement as to the resisting powers of the older eggs. It shows the percentage of these eggs which succeeded in hatching in spite of the very unnatural conditions (stagnant water, possible overcrowding, etc.) in which they were placed. Incidentally may be noticed the very deadly effect of "di-methyl," to which attention is now to be drawn.

II. Effect of Indicators on Pleuronectes and Echinus.

The effects which di-methyl-amido-azo-benzol and phenol-phthalein produce upon living organisms, as illustrated by the eggs of Pleuronectes and *Echinus esculentus*, were accidentally discovered in the course of these experiments. These indicators were originally added to the contents of some of the

* 'Arch. f. Entwickelungsmechanik,' vol. 7, p. 631.

tumblers, to show any changes in reaction that might take place during growth. When it was observed that they had a specific action upon the eggs, a series of experiments was undertaken with them, the results of which are shown in Tables VII—X. In all the experiments, except those recorded in Table X, two drops of the indicator were added to 200 c.c. of sea-water, or sea-water plus varying quantities of alkali, in a tumbler. Table X records the results of varying the amount of phenol-phthalein employed. It will be seen that, although the indicators were made up in alcoholic solution, the amount of alcohol added in each case to 200 c.c. of liquid, was quite insufficient to materially influence the result.

The experiments without indicators, recorded in Tables VIII and IX, are included for purposes of comparison.

It will be observed from the figures obtained that dimethyl is very deadly to the eggs of Pleuronectes and phenol-phthalein innocuous, while the opposite holds good with Echinus, the dimethyl having, if anything, a favouring effect on growth, and the phenol-phthalein being very injurious. Before killing, phenol-phthalein appears to be very effective in producing irregular divisions. The dimethyl was readily absorbed as such by both the organisms, staining them a deep yellow, so there can be no question as to its having thoroughly penetrated the tissues.

So far as can be ascertained, this specific action of indicators has not been noted before, and no explanation can be given of the fact of the different indicators affecting the two organisms in exactly opposite ways. Tadpoles in tap-water, to which the same, and even much larger amounts of these indicators had been added, appeared to be totally unaffected by either.

It might be of interest to repeat the experiments with other organisms, and with other organic compounds not known already to act as poisons.

Summary.

(1) The amount of variation from the normal concentration of hydrogen and hydroxyl ions in sea-water which the eggs of Pleuronectes will tolerate is very small.

(2) A disturbance of the equilibrium towards the acid side is much more fatal than the opposite.

(3) A progressive development of resistance to an unfavourable action of the environment takes place in proportion to the age of the eggs.

(4) Phenol-phthalein is deadly to the eggs of *Echinus esculentus*, but harmless to those of Pleuronectes, while dimethyl quickly kills the latter, and appears, if anything, to have a favourable influence upon the development of the former.

My best thanks, in conclusion, are due to Professor Herdman, F.R.S., to whose kindness I am indebted for the material for these experiments, and for permission to work at the station, and to Professor Moore, for his kind and valuable criticism and assistance throughout.

Table I.—Comparison of Percentages of Deaths in Fresh Eggs. Eggs of 2 days old and eggs of 10 days old, with varying quantities of decinormal NaOH and HCl.

	After 6 days. Fresh eggs.	After 7 days. 2 days old.	After 7 days. 10 days old.	After 10 days. Fresh eggs.	After 11 days. 2 days old.	After 11 days. 10 days old.
Control, 200 c.c. sea-water.	5·0	0	2·0	25·0	0	2·4 (9 days)
1·0 c.c. decinormal NaOH	4·3	0	4·2			
1·5 " "	9·6	0	2·9			
2·0 " "	25·0	0	0·9			
2·5 " "	18·2	2·6	3·0	31·8	2·6	
3·0 " "	27·3	12·7	2·5	31·8	14·5	2·5
4·0 " "	44·0	25·0	9·4	48·0	48·2	26·9 (10 days)
1·0 c.c. decinormal HCl...	10·0	3·3	2·2			
1·5 " " ...	0	8·0	4·8			
2·0 " " ...	8·6	3·0	1·5			
2·5 " " ...	19·0	20·0	3·3	28·6	26·2	
3·0 " " ...	33·3	39·3	1·7	33·3	42·4	4·6
4·0 " " ...	75·0	39·4	1·8	75·0	39·4	5·5
Average, exclusive of Control	22·9	12·8	3·2	41·4	28·9	9·9

Table II.—Actual Number of Deaths in Fresh Eggs. Eggs of 2 days old and eggs of 10 days old with larger quantities of acid and alkali than were employed in the experiments, the results of which are shown in Table I. 40 eggs in each tumbler.

	Fresh eggs.				2 days old.				10 days old.			
	1st day.	2nd day.	3rd day.	4th day.	1st day.	2nd day.	3rd day.	4th day.	1st day.	2nd day.	3rd day.	4th day.
Control	0	1	2	3	1	1	1	1	0	0	0	0
3 c.c. N/10 NaOH...	0	2	11	25								
4 " " ...	2	5	17	29								
5 " " ...	0	5	34	36	0	1	1	1	0	0	0	2
6 " " ...	?	29	36	all								
8 " " ...	?	10	all	—	0	?	?	28				
10 " " ...	—	—	—	—	—	—	—	—	0	1	2	less than 10
15 " " ...	—	—	—	—	—	—	—	—	0	2	3	less than 10
20 " " ...	—	—	—	—	—	—	—	—	0	2	?	26
30 " " ...	—	—	—	—	—	—	—	—	?	?	?	26
3 c.c. N/10 HCl......	0	1	1	2								
4 " "	0	0	1	3								
5 " "	5	8	18	30	3	9	20	23				

Strengths of 6, 8, 10, 15, 20 and 30 c.c. acid were also tried, all being fatal in less than 24 hours.

The figures for the 2nd, 3rd and 4th days are cumulative.

Table III.—Actual Number of Deaths among Fresh Eggs.

	No. of eggs.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.
Control	20	1	1	1	1	1	1	2	3	5
1·0 c.c. N/10 NaOH	23	0	0	0	1	1				
1·5 " "	21	0	1	1	2	2				
2·0 " "	20	1	4	4	5	5				
2·5 " "	22	4	4	4	4	4	6	6	6	7
3·0 " "	22	3	5	5	5	6	6	6	7	7
4·0 " "	25	3	8	9	11	11	11	12	12	12
1·0 c.c. N/10 HCl ...	20	1	2	2	2	2				
1·5 " "	21	0	0	0	0	0				
2·0 " "	23	2	2	2	2	2				
2·5 " "	21	2	3	3	3	4	5	5	5	6
3·0 " "	24	0	4	6	7	8	8	8	8	8
4·0 " "	20	3	9	15	15	15	15	15	15	15

The figures in each column in this and the following two tables give the *total* number dead by the day in question.

Table IV.—Actual Number of Deaths among Eggs 2 Days Old.

	No. of eggs.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.
Control	36	0	0	0	0	0	0	0	0	0	0
1·0 c.c. N/10 NaOH	28	0	0	0	0	0	0				
1·5 " "	23	0	0	0	0	0	0				
2·0 " "	32	0	0	0	0	0	0.				
2·5 " "	38	1	1	1	1	1	1	1	1	1	1
3·0 " "	55	0	0	0	0	4	7	7	7	7	8
4·0 " "	56	1	2	2	5	10	14	23	23	23	27
1·0 c.c. N/10 HCl ...	60	1	2	2	2	2	2				
1·5 " " ...	25	2	2	2	2	2	2				
2·0 " " ...	33	1	1	1	2	2	2				
2·5 " " ...	80	8	9	9	10	15	16	17	17	19	21
3·0 " " ...	33	10	10	10	10	13	13	14	14	14	14
4·0 " " ...	95	23	23	23	26	31	34	34	34	34	34

Table V.—Actual Number of Deaths among Eggs 10 Days Old.

	No. of eggs.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.
Control	245	0	0	1	4	4	5	6	6		
1·0 c.c. N/10 NaOH	165	0	2	4	6	6	7				
1·5 " "	105	0	1	1	1	1	3				
2·0 " "	110	1	1	1	1	1	1				
2·5 " "	100	0	0	1	1	1	3				
3·0 " "	80	0	0	0	1	1	2	2	2	2	2
4·0 " "	160	1	1	2	5	7	15	30	39	43	
1·0 c.c. N/10 HCl ...	135	0	1	1	2	2	3				
1·5 " " ...	105	0	0	2	2	4	5				
2·0 " " ...	155	0	0	0	0	0	1				
2·5 " " ...	120	0	1	2	3	3	4				
3·0 " " ...	175	0	3	3	3	3	3	3	5	6	8
4·0 " " ...	110	0	1	1	2	2	2	3	5	5	6

Table VI.—Percentages of 10-Days-old Eggs which succeeded in Hatching.

Control	98·4
+1·0 c.c. N/10 NaOH	96·4
1·5 "	99·0
2·0 "	99·1
2·5 "	99·0
3·0 "	98·7
4·0 "	95·6
+1·0 c.c. N/10 HCl	98·5
1·5 "	96·2
2·0 "	100·0
2·5 "	97·5
3·0 "	98·3
4·0 "	98·2
Dimethyl	39·7
Phenol-phthalein	98·8

Table VII.—Showing the Effect of Indicators upon the Eggs of Pleuronectes.

Batch.	Indicator.	No. of eggs.	Number dead at end of—										Percentage of dead at end of—									
			2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.	11 days.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.	10 days.	11 days.
Fresh eggs	Dimethyl	20	3	3	5	7	8	9	12	12	18	—	15	15	25	35	40	45	60	60	90	
	Phenol-phthalein	28	0	0	0	0	0	0	0	1	1	—	0	0	0	0	0	0	0	3·6	3·6	
	Control	20	1	1	1	1	1	1	2	3	5	—	5	5	5	5	5	5	10	15	25	
2 days old	Dimethyl	70	0	0	0	2	4	8	15	20	64	70	0	0	0	2·8	5·7	11·4	21·4	28·6	91·4	100
	Phenol-phthalein	50	1	1	1	1	1	1	1	2	2	3	2	2	2	2	2	2	2	4	4	6
	Control	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 days old	*Dimethyl	63	2	2	2	6	25	38	46	60	63	—	3·2	3·2	3·2	9·5	39·7	60·3	73	95·2	100	
	†Phenol-phthalein	83	0	0	1	1	1	1	2	2	2	—	0	0	1·2	1·2	1·2	1·2	2·4	2·4	2·4	
	Control	245	0	1	2	5	5	6	7	7	—	—	0	0·4	0·8	2·0	2·0	2·4	2·9	2·9		

* 25 of these eggs reached the larval stage.
† 82 " "

Table VIII (Brood 1).

Showing the Effect of Indicators upon Echinus Eggs in Solutions Containing Varying Quantities of NaOH in ·200 c.c Sea-water.

No.	Indicator.	Amount of N/10 NaOH.	After 4 hours.	17 h. 45 m.*	24 h. 50 m.	29 h. 15 m.	41 h. 45 m.	70 h. 30 m.	91 h. 15 m.	114 h. 0 m.
		c.c.								
1	None	0	4 cells...............	$1\frac{1}{2}$ Blastulæ ...	B., a few moving	All active	B., rapid movement	B., commencing G., mostly dead	Dead and degenerating	
2	"	0·2	"	$1\frac{3}{4}$ " ...	" "		" "	Early G., mostly dead	" "	
3	"	0·4	"	$1\frac{3}{4}$ " ...	" "			Commencing G., all dead, granular	" "	
4	"	0·8	4, 6, and 8 irreg.	$1\frac{7}{8}$ " ...	" "	" "	" "	G., active	Active, late G.‡	
5	"	1·4	4 irreg.	$1\frac{3}{4}$ " ...	B., motionless			"	$\frac{1}{2}$ dead, not so advanced as preceding	Very active
6	"	2·0	2, and a few 4 ...	$1\frac{7}{8}$ " ...	" "	" "	B. and some G.	" †	Dead, but better formed and more advanced than preceding	
7	Dimethyl	0	$\frac{4}{5}$ 4-celled, $\frac{1}{5}$ 8-celled	$1\frac{7}{8}$ " ...	B., some moving	" "	B., swimming.	Early G., dead, granular	Dead and degenerating	
8	"	0·2	" "	$1\frac{3}{4}$ " ...	" "			" "	" "	
9	"	0·4	4, and occasional 2 and 8	$1\frac{3}{4}$ " ...	" "			Early G., not very active	Dead	
10	"	0·8	4, and $\frac{1}{10}$ above 4	$1\frac{7}{8}$ " ...	Several moving	Some active some encapsuled (?)	" "	Early G., fairly active	More advanced than preceding, but dead	
11	"	1·4	4 irreg., some 8 and 12	$1\frac{3}{4}$ " ...	A few moving			Early G., slightly active	" "	Degenerating
12	"	2·0	4, 6, 8, and more	$1\frac{3}{4}$ " ...	" "	" "	Gastrulæ forming	" "	Well advanced, dead	Not yet degenerating

13	Phenol-phthalein	0	4, and $\frac{1}{3}$ 8	$1\frac{3}{4}$ Morulæ......	M. and $\frac{1}{30}$ B.	Same as before	Degenerated	Dead and degenerated		
14	,,	0·2	Irreg., a few 2, mostly 4	12 cells less divided than preceding	M.		,,	,, ,,		
15	,,	0·4	4 and over, several 2, irreg., and breaking up	Irreg. division, $1\frac{3}{8}$ M.	M., irreg.			,, ,,		
16	,,	0·8	Many, 3 irreg., a few 2	M. irreg., $1\frac{3}{4}$...	,, ,,	,, ,,		Dead		
17	,,	1·4	4, $\frac{1}{3}$ more, very irreg.	M., $1\frac{3}{4}$ more cells than preceding	,, ,,			,,		
18	,,	2·0	Very irreg., 2, 3, and 4	,, ,,	Several badly-formed B.	,, ,,	B., motion-less	,,		

* Measurements arbitrary by linear marks on paper. † Small amount of formalin added by mistake after examination.

‡ Bottle unfortunately broken after examination.

Table IX.—Effect of Indicators on Echinus Eggs in Solutions containing Varying Quantities of KOH in 200 c.c. Sea-water.

(Brood 2.)

No.	Indicator.	Amount of N/10 KOH.	After 4 hrs. 30 mins.	12 hrs. 30 mins.	24 hrs. 30 mins.
		c.c.			
1	None	0	2's	Morulæ and early blastulæ, 17*	Dead
2	„	1·0	2, occasional 3 and 4	Early blastulæ, more advanced	Blastulæ, some early gastrulæ
3	„	1·5	„ „	Blastulæ, 26	Dead
4	„	2·0	„ „	Blastulæ, few developed	Commencing gastrulæ, dead
5	„	3·0	Nearly all 1's, irregular, granular	No morulæ, irregular 2's and up to 16's	Poorly developed, some morulæ, dead
6	„	4·0	Irregular division, very few cells visible	Broken up	
7	„	5·0	None divided	„	
8	Dimethyl	0	2, some incomplete 4	Morulæ and early blastulæ, 18	Blastulæ, dead
9	„	1·0	2's	Blastulæ, 28	Blastulæ and occasional early gastrulæ, dead
10	„	1·5	2, a few incomplete 3	„	„ „ „
11	„	2·0	2, irregular division into 3	Slightly larger than preceding	„ „ „
12	„	3·0	Same as 5	Irregular, no blastulæ yet formed	Morulæ or earlier stage, dead
13	„	4·0	Irregular and breaking up	16 cells and less, irregular	Unsegmented or broken up
14	„	5·0	1's, a few irregular divisions	Irregular and broken up	
15	Phenol-phthalein	0	2, a few 4	Morulæ and less, 16 irregular	Morulæ, dead
16	„	1·0	2, 4, and incomplete further divisions	Early blastulæ, 20	„ „
17	„	1·5	Many 1, some 2, and some incomplete 4	„ „ 12	Morulæ or earlier stages, dead
18	„	2·0	2 and irregular 3 and 4	„ „ 16	Better than last
19	„	3·0	Same as 5	Few morulæ, mostly 2, 3, 8, and 16	Same as last but one
20	„	4·0	Same as 13	Very irregular, but nearly all 1	Same as before
21	„	5·0	Same as 7	Single cells, breaking up	

* Numbers in this column give number of cells counted in the circumference of the blastula.

Table X.—Effect on Development of Echinus Eggs of 0·5 Per Cent. Alcoholic Solution of Phenol-phthalein in Varying Strengths, added in each Case to 200 c.c. Sea-water.

(Brood 3.)

No.	Amount of indicator.	3 hrs. 30 mins.	19 hrs.	47 hrs. 30 mins.	71 hrs.	114 hrs.
26	c.c. 0·05	Some complete 2, some incomplete 3 and 4	$\frac{1}{5}$ blastulæ, $\frac{1}{5}$ morulæ, rest earlier	Morulæ and a few developing blastulæ	Not well developed	Degenerating blastulæ
27	0·1	Mostly 2, also 3 and 4...	$\frac{1}{3}$ at 2, rest morulæ and blastulæ about equal	Slightly earlier than last	... ...	$\frac{3}{4}$ countable, 2 morulæ, a few blastulæ
28	0·15	2, several 4, some irregular	No blastulæ, $\frac{1}{2}$ badly-formed morulæ, rest 2, 3, 4, etc.	Early morulæ, a few still remain unsegmented	Only slightly developed	Less advanced and more irregular than last
29	0·2	... ...	Less advanced	A few morulæ, about $\frac{1}{2}$ still unsegmented	... ...	Singles to early morulæ, nearly all countable
30	0·3	... ...	$\frac{9}{10}$ not beyond 2's, rest irregular morulæ	Still more singles than last	Mostly 1, 2 and 3.........	Singles, 2, 4, 6, $\frac{1}{10}$ early morulæ, less advanced than last
31	0·4	1's, a few incomplete 2	$\frac{9}{10}$ single, scarcely any 2, rest irregular 4, 8 and 12	$\frac{3}{4}$ singles, rest early divisions, irregular	... ...	Less advanced still, $\frac{1}{20}$ early morulæ, nearly all single but some irregular attempts at division